Aspects of Microbiology 5

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Nelson

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# 1 Introduction

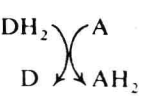
Energy conservation in bacteria, as in higher organisms, occurs principally via the synthesis of adenosine-5′-triphosphate (ATP) from adenosine-5′-diphosphate (ADP) and inorganic phosphate:

$$H^+ + ADP^{3-} + HPO_4^{2-} \rightleftharpoons ATP^{4-} + H_2O$$

Since the hydrolysis of ATP under standard conditions releases a moderate amount of free energy ($\Delta G^{\theta'} = -31.0\,kJ.mole^{-1}$), its synthesis requires a similar amount of energy ($\Delta G^{\theta'} = +31.0\,kJ.mole^{-1}$), the latter being provided by specific metabolic reactions within the cell.

Bacteria use three quite distinct methods for synthesizing ATP: substrate-level phosphorylation, respiratory chain (or oxidative) phosphorylation and photosynthetic (or photo) phosphorylation. Each method involves one or more oxidation-reduction (redox) reactions, but the way in which these exergonic reactions are coupled to the endergonic condensation of ADP and phosphate is fundamentally different in substrate-level phosphorylation compared with the other two processes.

During a redox reaction, reducing equivalents (H, $H^-$ or $e^-$) are spontaneously transferred from a compound which has a tendency to donate them (the reducing half of a low redox potential couple) to a compound which has a tendency to accept them (the oxidizing half of a higher redox potential couple) e.g.




According to equilibrium thermodynamics, the amount of free energy released by this reaction under standard conditions is determined by the difference between the redox potential ($E'_\theta$) of the donor couple ($D/DH_2$) and the acceptor couple ($A/AH_2$), according to the equation:

$$\Delta G^{\theta'} = -n.F.\Delta E'_\theta$$

where n is the number of electrons transferred, F is the Faraday constant ($96.6\,kJ.volt^{-1}.equiv^{-1}$) and $\Delta E'_\theta$ is the difference in standard redox potential ($E'_{\theta_{ox}} - E'_{\theta_{red}}$; V or mV). An exergonic redox reaction of this type can therefore be coupled to the performance of useful work, such as the formation of an energy rich compound or the generation of a membrane-associated concentration or charge gradient, both of which can subsequently be used to drive ATP synthesis.

Thus some types of substrate level phosphorylation entail the oxidation of an organic substrate (e.g. pyruvate or 3-phosphoglyceraldehyde) by an appropriate endogenous oxidant such as $NAD^+$ to generate a non-phosphorylated intermediate with a high free energy of hydrolysis. This subsequently undergoes phosphate substitution to yield an energy-rich acyl phosphate (such as acetyl phosphate or 1,3-bis-phosphoglycerate; $\Delta G^{\theta'} \geq -43.9\,kJ.mole^{-1}$) which finally

donates a phosphoryl group ($-PO_3^{2-}$) to ADP to form ATP. Substrate-level phosphorylation is thus a scalar (spatially-directionless) series of reactions in which chemical group transfer is catalyzed by essentially soluble cytoplasmic enzymes via the sequential stoichiometric formation of covalent intermediates. In contrast, oxidative and photosynthetic phosphorylation are membrane-bond vectorial (spatially-oriented) processes which occur via a series of sequential oxidation-reduction reactions (respiration and photosynthetic electron transfer) that involve several types of spatially-organized redox carriers (the respiratory chain and the photosynthetic electron transfer system). No covalent, energy-rich intermediates have been detected during either of these processes, and energy transfer between the redox systems and the enzyme complex which is responsible for ATP synthesis (the ATP phosphohydrolase or ATPase-ATP synthetase) appears to be effected via energized protons, i.e. an electrochemical gradient of $H^+$. The latter can also drive other energy-dependent membrane reactions such as reversed electron transfer, cell motility and some forms of solute transport.

Substrate-level phosphorylation is the only method of ATP synthesis that is available to a few obligately anaerobic chemoheterotrophs and, under certain oxygen-deficient growth conditions, to some facultative anaerobes. In both cases it is associated with a metabolic process known as fermentation in which reducing equivalents conserved during catabolism as NADH or as other reduced cofactors are ultimately transferred to one or more endogenous organic oxidants. Since the amount of free energy liberated during fermentation is usually small, the ATP yield during substrate-level phosphorylation is correspondingly low (e.g. the homolactic fermentation of one molecule of glucose to two molecules of lactate by members of the genus *Streptococcus* yields only two molecules of ATP net, and some closely-related fermentations yield only one). In contrast, substrate-level phosphorylation is responsible for only a small fraction of the total ATP which is synthesized by bacteria which carry out respiration (most chemoheterotrophs, all chemolithotrophs and some facultative phototrophs) or photosynthetic electron transfer (most phototrophs).

## Respiration

Aerobic respiration in chemoheterotrophs and facultative phototrophs is characterized by the transfer of reducing equivalents from an organic donor, principally NADH (but also other reductants including succinate, lactate and methanol), to molecular oxygen. Since NADH oxidation occurs over a large redox potential span, ($E_0' NAD^+/NADH + H^+ = -320\,mV, \frac{1}{2}O_2/H_2O = +820\,mV; \Delta E_0' = +1140\,mV$), both the free energy change and the ATP yield are high (e.g. the complete oxidation of one molecule of glucose to carbon dioxide and water probably yields up to 38 molecules of ATP net in some organisms). These aerobic respiratory chains contain diverse redox carriers which include flavoproteins (Fp), iron-sulphur proteins (Fe-S), quinones and cytochromes (iron-containing haemoproteins); the first two usually form the dehydrogenases which catalyse the initial oxidation of the donor, whereas specialized autoxidizable cytochromes comprise the one or more oxidases which catalyse the terminal reduction of oxygen to water (Fig. 1.1a).

Many facultatively anaerobic and obligately anaerobic chemoheterotrophs replace oxygen with alternative acceptors, and hence catalyse anaerobic respiration. These acceptors include various oxy-anions of nitrogen and sulphur,

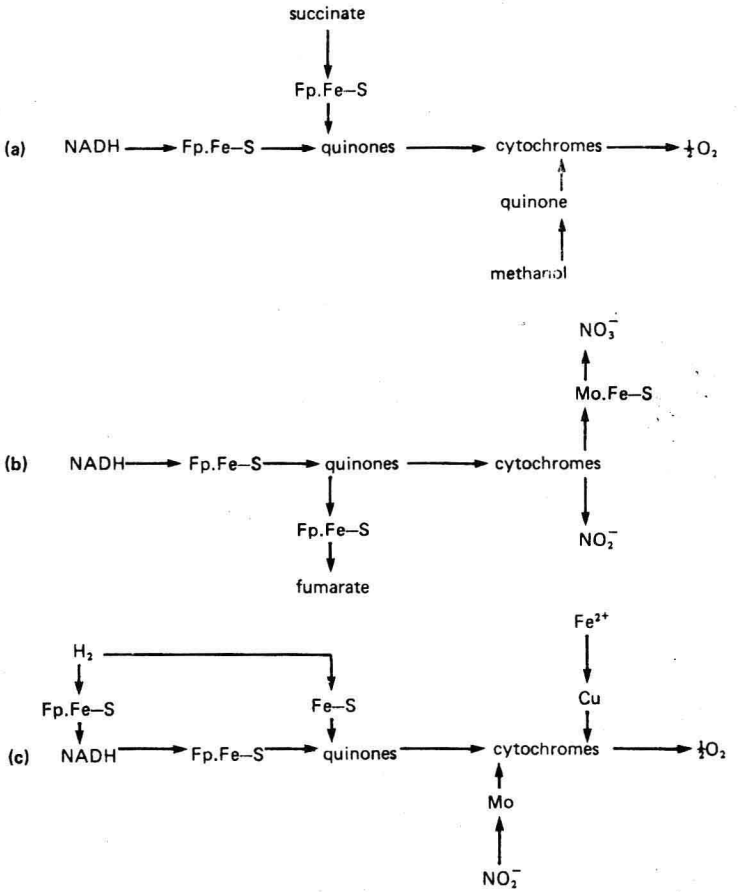



**Fig. 1.1** Some examples of respiration in (a) aerobic chemoheterotrophs and facultative phototrophs, (b) anaerobic chemoheterotrophs, and (c) chemolithotrophs.

$Fe^{3+}$ and organic compounds such as fumarate, carbon dioxide and trimethylamine-N-oxide; their reduction is said to be dissimilatory since the products are ultimately released into the environment. The redox potentials of these acceptors are very wide-ranging (e.g. $E'_0 SO_3^{2-}/S^{2-} = -116\,mV$, $N_2O/N_2 = +1355\,mV$) and hence the ATP yield from anaerobic respiration is extremely varied. However, except for the reduction of $Fe^{3+}$ and some nitrogen compounds, the yield is generally much lower than from aerobic respiration, although higher than from fermentation. Anaerobic respiratory chains contain the same types of redox carriers as those present in aerobic systems, except that the cytochrome oxidases are replaced by appropriate reductases (Fig. 1.1b). Some of the latter are novel redox carriers (e.g. molybdo-proteins and copper proteins), but the majority are specialized flavoproteins, iron-sulphur proteins or cytochromes.

Chemolithotrophs (chemoautotrophs) principally oxidize inorganic donors using mainly oxygen, but occasionally nitrate, as the terminal acceptor; the donors include hydrogen, various nitrogen and sulphur compounds, $Fe^{2+}$ and, paradoxically, carbon monoxide. Since the redox potentials of these donors cover a very wide range (e.g. $E'_0\ CO_2/CO = -540\,mV$, $2H^+/H_2 = -420\,mV$, $Fe^{3+}/Fe^{2+} = +780\,mV$), the free energy changes and the ATP yields associated with this type of respiration also vary tremendously; however, except for the oxidation of hydrogen and probably also carbon monoxide, they are generally fairly low. Specially adapted enzymes, many of which resemble the corresponding anaerobic reductases, catalyze the initial oxidation of the inorganic donors (Fig 1c).

Each of these three types of respiration is characterized by the transfer of reducing equivalents in the direction of increasingly positive redox potential, with the concomitant release of free energy, (it should be noted that under non-standard conditions $E'_0$ is replaced by $E_h$, the actual redox potential, and that $\Delta G^{0'}$ is replaced by $\Delta G$, the actual free energy change; under certain circumstances the values of $E_h$ and $\Delta G$ may differ significantly from those of $E'_0$ and $\Delta G^{0'}$ respectively). It is possible for respiratory chains to transfer energy in the opposite direction provided that energy is put into the system (reversed respiration or reversed electron transfer). This phenomenon is particularly crucial to chemolithotrophs since these organisms require NAD(P)H for carbon dioxide assimilation. Reversed electron transfer from higher redox potential inorganic donors to $NAD(P)^+$ is driven by the proton or charge gradient generated during forward electron transfer from the same donors to oxygen or nitrate.

## Photosynthesis

The overall reaction of photosynthesis in photoautotrophs may be described by the equation:

$$2H_2A + CO_2 \xrightarrow{\text{light energy}} (CH_2O) + H_2O + 2A$$

In blue-green bacteria, as well as in algae and green plants, $H_2A$ is water and the reductive assimilation of carbon dioxide is accompanied by the release of molecular oxygen (oxygenic photosynthesis). However, many species of purple and green bacteria replace water with other inorganic reductants (e.g. $H_2$, $S^{2-}$, $S_2O_3^{2-}$), and others, photoheterotrophs, replace both water and carbon dioxide with partially reduced carbon compounds such as succinate or malate; oxygen is not released by any of these organisms (anoxygenic photosynthesis).

Two types of photosynthetic electron transfer and phosphorylation, cyclic and non-cyclic, are found in the majority of phototrophs (Fig. 1.2). The former is independent of exogenous reductants or oxidants and its sole function is to conserve light energy as a proton/charge gradient and hence as ATP (some species of purple bacteria will also synthesize inorganic pyrophosphate, $\Delta G^{0'} = +21.9\,kJ.mol^{-1}$, from two molecules of orthophosphate). In the first stage of this process, electro-magnetic radiation is absorbed by various specialized photopigments which include (bacterio) chlorophylls, (bacterio) pheophytins, carotenoids and, in the blue-green bacteria, various phycobiliproteins. This leads, via a series of complex photochemical reactions, to the generation of a low redox potential reductant (a reduced iron-sulphur protein in green and blue-green bacteria, and a novel quinone-iron complex in purple bacteria; $E'_0 \leq -160\,mV$) and

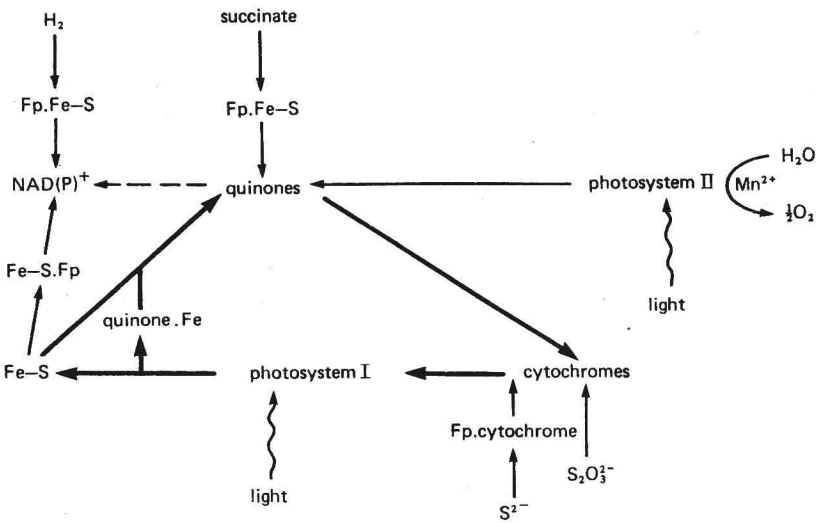



**Fig. 1.2** Photosynthetic electron transfer. Cyclic electron transfer (→), the additional steps of non-cyclic electron transfer (→), reactions driven by the proton/charge gradient (⇢). See text for further explanations.

a high redox potential oxidant (oxidized (bacterio) chlorophyll; $E'_\theta = +250\,mV$). The transfer of reducing equivalents from the former to the latter occurs via a conventional quinone-cytochrome system and leads to the release of a medium amount of free energy and hence to a moderate ATP yield. In contrast, the principal function of non-cyclic electron transfer is to reduce $NAD(P)^+$ using an exogenous donor, the resultant NAD(P)H being used for the assimilation of carbon dioxide and for various other metabolic reactions. Since the exogenous reducing couple, with the exception of $2H^+/H_2$, has a higher redox potential than the $NAD(P)^+/NAD(P)H$ couple (e.g. $E'_\theta S_0/S^{2-} = -99\,mV$, fumarate/succinate $= +30\,mV$, $\frac{1}{2}O_2/H_2O = +820\,mV$), the reduction of $NAD(P)^+$ is energy-dependent. In this case, however, the reaction is driven either by solar radiation (green and blue-green bacteria) or by the proton/charge gradient generated via cyclic electron transfer (purple bacteria). In the blue-green bacteria the extremely high redox potential of the oxygen/water couple necessitates the involvement in non-cyclic electron transfer of a high redox potential photo-pigment system (photosystem II) in addition to the ubiquitous low redox potential system (photosystem I). Since the redox spans of the two photosystems overlap, the excess energy can be used to synthesize a small amount of ATP. Photosystem II contains similar photopigments to those present in photosystem I, plus phycobiliproteins and manganese proteins which assist with the release of oxygen. The oxidation of inorganic reductants in other phototrophs is catalysed by specially adapted cytochromes or novel flavo-cytochromes.

The respiratory chains and photosynthetic electron transfer systems of bacteria, like those of higher organisms, contain both organic redox centres (flavoproteins and quinones) and metal-containing redox centres (cytochromes, iron-sulphur

proteins, molybdoproteins, copper proteins and manganese proteins). The organic centres are of relatively fixed redox potential and transfer hydrogen atoms, whereas the metal centres can often span a wide range of redox potential and catalyse electron and oxygen atom transfer. These two types of redox centre are therefore associated with the oxidation-reduction of organic and inorganic substrates respectively.

A few species of red halobacteria catalyse photophosphorylation in the absence of either bacteriochlorophyll or conventional redox carriers. Instead they use a novel photopigment, bacteriorhodopsin, which is similar in structure to the pigment rhodopsin (visual purple) that functions in vertebrate vision. Bacteriorhodopsin harnesses solar energy directly to the formation of a proton/charge gradient.

Although most photosynthetic bacteria are obligate phototrophs, the halobacteria and some species of purple and blue-green bacteria are facultative and hence catalyse respiratory chain phosphorylation under aerobic conditions. A few species can also grow anaerobically in the dark, conserving energy via substrate-level phosphorylation during the fermentation of glucose or pyruvate.

## Respiratory chain and photosynthetic phosphorylation

The free energy released by the redox reactions of respiration and photosynthetic electron transfer is conserved as ATP via the membrane-bound ATP phosphohydrolase. The latter consists of two multipeptide assemblies, termed $BF_0$ and $BF_1$ (where BF stands for bacterial coupling factor). $BF_1$ is a hydrophilic polypeptide complex which is located on the cytoplasmic surface of the energy-coupling membrane and is responsible for ATP synthesis and hydrolysis by the overall complex. It is easily detached from $BF_0$, but in its soluble form it catalyses only ATP hydrolysis. In contrast, $BF_0$ is an assembly of hydrophobic polypeptides and proteolipids which forms an intrinsic part of the membrane and probably facilitates the utilization by $BF_1$ of the proton/charge gradient generated by the various redox systems.

The stoichiometry of ATP synthesis during oxidative and photosynthetic phosphorylation is expressed as the ATP/O($ATP/2e^-$) quotient or P/O($P/2e^-$) quotient (mole ATP synthesized or phosphate esterified).(g-atom O or mole of alternative two-electron acceptor reduced)$^{-1}$.

The determination of the mechanism of membrane-associated energy transduction is one of the major problems of contemporary biochemistry. Although it is now generally accepted that energized protons are the primary intermediates in this process, there is still considerable controversy regarding the generation, location and utilization of these protons. Two major hypotheses, the chemiosmotic and localized proton hypotheses, have been proposed and are currently receiving considerable experimental attention.

**The chemiosmotic hypothesis** The major tenets of this hypothesis, which was first proposed by Mitchell in 1961, are that oxidative and photosynthetic phosphorylation require (i) a proton-translocating redox system, (ii) a proton-translocating ATP phosphohydrolase, and (iii) a passive coupling membrane which is impermeable to ions, particularly $H^+$ and $OH^-$, except via specific exchange-diffusion systems. Energy transduction thus occurs via a proton current which circulates through the insulating membrane and the adjacent bulk aqueous phases (Fig. 1.3a);

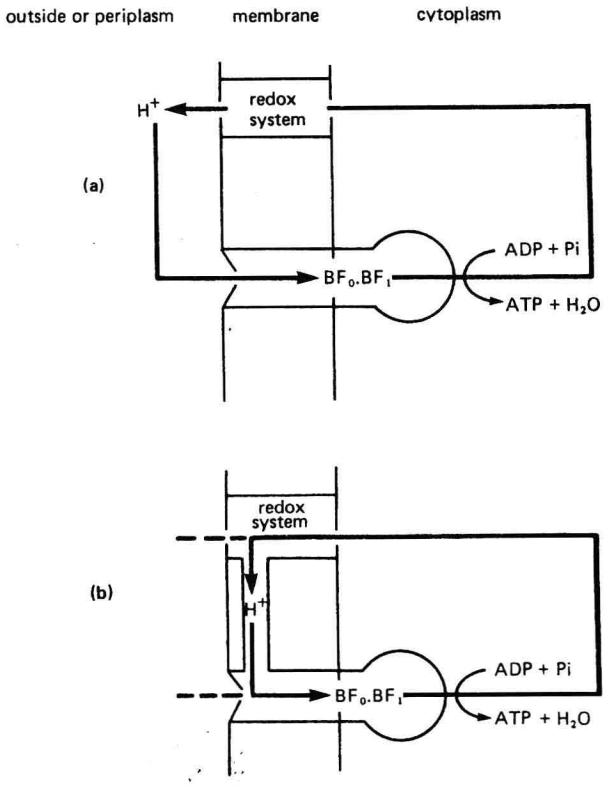



**Fig. 1.3** The proton current according to (**a**) the chemiosmotic hypothesis, and (**b**) the localized proton hypothesis.

since the latter are in equilibrium, energy storage is transmembrane rather than intramembrane, and takes the form of a delocalized electrochemical potential difference of protons or protonmotive force ($\Delta p$ or $\Delta_{\tilde{\mu}H^+}$; mV). This is variably composed of a chemical potential difference $\Delta pH (pH_{out} - pH_{in})$ and an electrical potential difference or membrane potential ($\Delta\psi$) according to the relationship:

$$\Delta p = \Delta\psi - Z.\Delta pH$$

where $Z(\equiv 2.303\,RT/F)$ has a value of approximately 60 at 25° and serves to convert $\Delta pH$ into electrical units. The hypothesis is said to be chemiosmotic since it involves both the transfer of chemical groups ($H$, $H^-$, $e^-$, $O^{2-}$) within the membrane and the transport of a solute ($H^+$) across the membrane.

According to the chemiosmotic hypothesis, respiration and photosynthetic electron transfer are described by the general equation:

$$DH_2 + A + zH^+_{(in)} \rightleftharpoons zH^+_{(out)} + AH_2 + D$$

where z is numerically equal to the $\rightarrow H^+O(\rightarrow H^+/2e^-)$ quotient (g-ion $H^+$.

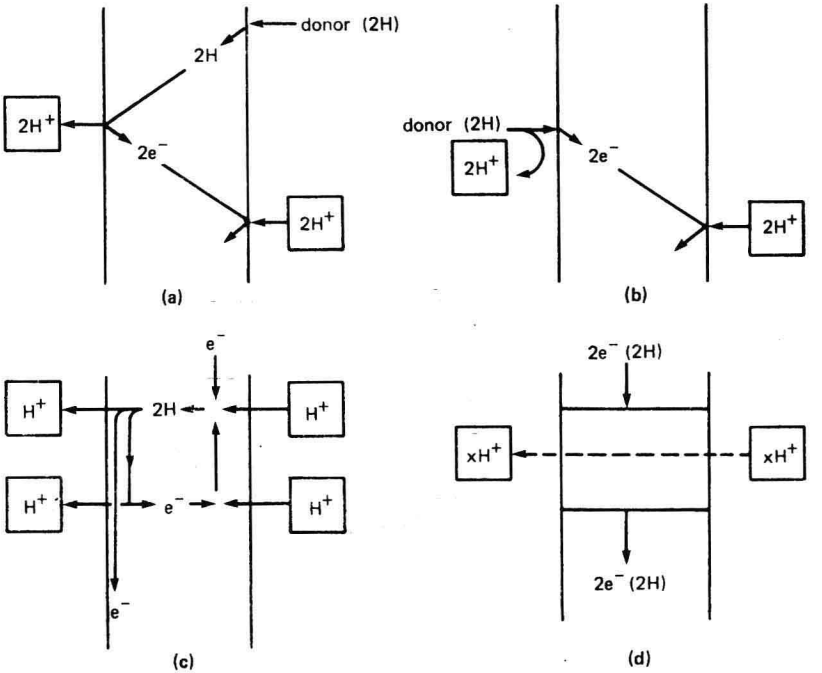



**Fig. 1.4** Protonmotive redox reactions. (**a**) loop, (**b**) arm, (**c**) cycle and (**d**) pump.

g-atom O or mole of alternative two-electron acceptor reduced$^{-1}$). Redox-linked electrogenic (charge producing) proton translocation is thought to occur at organic centre/metal centre junctions, the centres being spatially and sequentially organized within the membrane such as to catalyse the release of protons at the outer surface and the uptake of protons at the inner surface (Fig. 1.4). These various protonmotive redox arms, loops and cycles may also be replaced by or supplemented with proton pumps in which redox-linked (or, in the case of bacteriorhodopsin, redox-independent) conformational changes in specific membrane proteins lead to changes in the pKa of appropriately located carboxyl or amino groups, and hence to the assymetric uptake and release of protons across the membrane (so-called membrane Bohr effects).

Similarly, ATP synthesis may be described by the equation:

$$H^+ + ADP^{3-} + HPO_4^{2-} + xH^+(out) \rightleftharpoons xH^+(in) + ATP^{4-} + H_2O$$

where x is numerically equal to the $\rightarrow H^+/ATP$ ($\rightarrow H^+/P$) quotient (g-ion $H^+$.mole ATP synthesized or phosphate esterified$^{-1}$); the other proton is scalar rather than vectorial and reflects ionization changes during the reaction. The ATP/O (ATP/2e$^-$) quotient is therefore equal to the $\rightarrow H^+/O$ ($\rightarrow H^+/2e^-$) quotient divided by the $\rightarrow H^+$/ATP quotient. Chemiosmosis envisages that ATP synthesis is initiated by the movement of $ADP^{3-}$ and phosphate to the active site of $BF_1$, with the concomitant release of $2H^+$ into the internal compartment. $O^{2-}$ is

then transferred from phosphate to the $2H^+$ which enters from the external compartment via $BF_0$ under the driving force of the $\Delta p$, and the resultant highly active phosphorylium group ($P^+O_3^{2-}$) is attacked by $MgADP^-$ to form $ATP^{4-}$ the latter, plus water, is finally released into the internal compartment.

The chemiosmotic hypothesis makes several other important predictions with respect to the mechanism of energy transduction: (i) since ATP synthesis (and some other energy-dependent membrane processes) are envisaged to be independent of covalent intermediates and to be driven by a delocalized proton/charge gradient, it should be possible experimentally to drive these reactions at the expense of artificially-imposed $\Delta pH$ and/or $\Delta\psi$; (ii) energy transduction should only occur in membranes which effectively separate the external and internal bulk aqueous phases, i.e. the membranes should be topologically closed; (iii) the rate of respiration and photosynthetic electron transfer should be controlled by the back pressure of $\Delta p$, thus preventing wasteful and unnecessary redox activity (respiratory control and photosynthetic control); (iv) since $\Delta p$ is inversely proportional to the rate of proton conductance through the coupling membrane, any compound which increases the latter should thus dissipate $\Delta p$ and effectively inhibit energy transduction whilst stimulating the redox reactions (such protonophores thus act as uncoupling agents); and (v) in order to prevent respiration and photosynthetic electron transfer from building up an osmotically-disruptive $\Delta pH$, the exchange-diffusion systems in the membrane should catalyse the uptake of protons either with anions or in exchange for cations (i.e. $H^+$.anion symport and $H^+$.cation antiport respectively). Such systems, in association with cation uniports, would also facilitate the energy-dependent import and export of metabolically important solutes.

**The localized proton hypothesis** This hypothesis, which was developed by Williams, differs from chemiosmosis in that it considers the energized protons to be localized (i.e. intramembrane or trans-interface) rather than delocalized (Fig. 1.3b). Furthermore, the postulated absence of a significant osmotic component (i.e. the transport of $H^+$ across the membrane) means that this hypothesis is satisfied by the presence of a membrane plus a cytoplasmic aqueous phase, whereas chemiosmosis additionally requires the external aqueous phase. The localized proton hypothesis envisages that during respiration and photosynthetic electron transfer, protons and electrons are separated at organic centre/metal centre junctions, and that subsequent proton diffusion between the redox and ATP phosphohydrolase systems is not only extremely rapid but also under strict kinetic control, such that the protons equilibrate only relatively slowly with the adjacent aqueous phases. Redox-linked conformational changes in $BF_0.BF_1$ are thought to exclude water from the active site of the ATP phosphohydrolase and allow the controlled access of phosphate, ADP and protons, the binding of the protons leading to further conformational changes which facilitate the removal of water from ADP and phosphate, and hence the synthesis of ATP. This hypothesis thus stresses the importance of water in oxidative and photosynthetic phosphorylation, and emphasizes the need for proton-binding rather than proton transport (the absence of osmosis implies that ATP synthesis should be possible in non-vesicular membrane preparations). Furthermore, it makes no predictions with respect to the stoichiometry of proton movement (i.e. $\rightarrow H^+/2e^-$ and $\rightarrow H^+/ATP$ quotients) and claims that the only obligatory stoichiometry is that which is observed experimentally for overall ATP synthesis (i.e. the $ATP/2e^-$ quotient). Reversed electron transfer and uncoupling of

energy transduction are thought to occur via somewhat analogous mechanisms to those of chemiosmosis, the former being driven by the back pressure of the localized proton concentration, the intramembrane dissipation of which by protonophores leads to uncoupling.

Since the results of most studies of bacterial respiration and photosynthesis have been interpreted almost entirely in terms of chemiosmosis, the latter will be used in this book as the working mechanism of membrane energy transduction. It will be seen, however, that although some systems are probably chemiosmotic, others can be interpreted just as well in terms of the localized proton hypothesis, and in a few cases the latter appears to explain the experimental observations more successfully.

## The coupling membrane

The ability of most bacteria to react positively or negatively to the Gram stain reflects basic differences in the structure and composition of their cell envelope. In Gram positive bacteria, all of which are chemoheterotrophs, the cytoplasm is usually bounded by an 8 $\mu$m thick membrane (the plasma membrane or cytoplasmic membrane) that is responsible both for energy coupling and for the compartmentation and transport of solutes. This membrane is surrounded by, and tightly connected to, a 10 to 80 $\mu$m thick rigid cell wall which is composed principally of peptidoglycan and is occasionally covered with a layer of slime.

The cell envelope of Gram negative bacteria (i.e. many chemoheterotrophs, especially those with specialized metabolic properties, and all chemolithotrophs and phototrophs) is a very much more complex structure which consists of three layers: an outer membrane, a thin (2 $\mu$m) and net-like peptidoglycan layer, and an inner membrane. The first two form a relatively rigid exoskeleton which is separated from the more flexible inner membrane by the periplasmic space. The 8 $\mu$m thick outer membrane, which is composed of phospholipid abundantly interspersed with protein, lipoprotein and lipopolysaccharide, has a limited capacity to control solute uptake; it thus provides a barrier to potentially toxic materials such as detergents and antibiotics, whilst allowing the free passage of many small molecules either by simple diffusion or via transmembrane pores. The periplasmic space is 3 to 4 $\mu$m wide and contains various hydrolytic enzymes, solute-binding proteins and, less frequently, certain redox carriers. The inner (plasma) membrane has similar structural and functional properties to its counterpart in Gram positive bacteria.

In many organisms the plasma membrane simply follows the inner contours of the cell, but in others it exhibits a more complex shape and can extend into the cytoplasm to form the intracytoplasmic membrane. Extensive vesicular and lamellar intrusions of various types, including layers of concentric or parallel membranes, are exhibited by some obligately aerobic chemoheterotrophs (particularly those which grow on one-carbon compounds or which fix atmospheric nitrogen) and by many chemolithotrophs and purple phototrophs. Relatively large closed vesicles (chlorosomes; originally called chlorobium vesicles) are found in green phototrophs, where they are arranged around the periphery of the cytoplasm and may be loosely attached to the plasma membrane. Uniquely, a second and quite separate intracytoplasmic membrane is dispersed throughout the cytoplasm of the blue-green bacteria where it is organized into stacks of flattened sacs (thylakoids) that are covered by small granules (phycobilisomes) which contain the light-harvesting apparatus.

In most bacteria the coupling membrane consists of a classical tail-to-tail phospholipid bilayer interspersed with protein. The phospholipids are mostly phosphatidylglycerol and phosphatidylcholine in Gram-positive bacteria, and phosphatidylethanolamine and phosphatidylcholine in Gram-negative organisms. The coupling membrane functions optimally in a fairly fluid liquid crystalline state, and its composition is therefore geared to maintaining this state. Thus, since the fluidity of the membrane is chiefly determined by the melting points of the fatty acid components of the phospholipids, the membranes of bacteria which grow at high temperatures (thermophiles) contain mainly longer chain saturated fatty acids with high melting points, whereas the converse is true in low temperature organisms (psychrophiles). Furthermore, bacteria which grow at extremes of salinity, pH and/or temperature (i.e. halophiles, acidophiles, aklaliphiles, thermoacidophiles and thermoalkaliphiles) contain significant amounts of unusual phospholipids

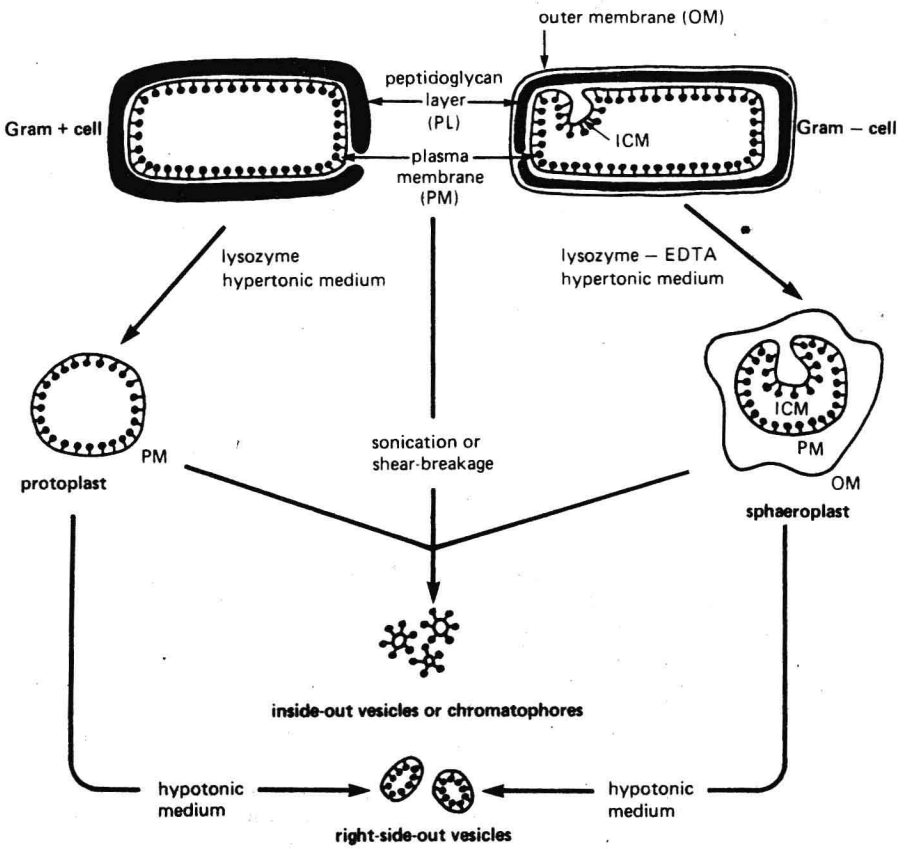



**Fig. 1.5** The preparation of protoplasts, sphaeroplasts and membrane vesicles. Abbreviations: OM, outer membrane; PL, peptidoglycan layer; PM, plasma (inner) membrane; ICM, intracytoplasmic membrane (shown here as a single vesicular intrusion into a phototrophic cell). After Konings (1977).

and/or more complex lipids (glycolipids, sulpholipids, diglycerol tetra-ethers). Indeed, some thermoacidophiles go so far as to abandon the bilayer membrane in favour of a complex-lipid monolayer. In each case the alteration in membrane composition and/or structure reflects an attempt to maintain the required fluidity and integrity in the face of a hostile environment. The protein components of the coupling membrane are either predominantly hydrophilic proteins which are associated with the periphery of the membrane (extrinsic proteins, e.g. $BF_1$ and some redox carriers) or predominantly hydrophobic proteins which are integral components of the membrane and sometimes completely span the latter (intrinsic proteins, e.g. $BF_0$, transport permeases and most redox carriers).

Exposure of whole cells to the enzyme lysozyme under the appropriate conditions causes degradation of the peptidoglycan layer and hence the conversion of Gram-positive and Gram-negative bacteria into osmotically fragile protoplasts and sphaeroplasts respectively (Fig. 1.5). Both of these have been used to investigate energy transduction, but more recently greater emphasis has been placed on the use of membrane vesicles. The latter are usually prepared either by exposing protoplasts and sphaeroplasts to ultrasound or hypotonic conditions, or by shearing whole cells under pressure (e.g. using a French pressure cell). The vesicles produced by osmotic shock are relatively large (0.8 to 1.1 $\mu$m diameter), do not readily hydrolyse ATP, and are called right-side-out vesicles since the orientation of the membrane is the same as in the parent protoplasts or sphaeroplasts, i.e. $BF_1$ is on the inner surface. In contrast, the vesicles prepared by sonication or shearing are smaller ($\leq 0.1\,\mu$m diameter), readily hydrolyse ATP, and are termed inside-out vesicles (or chromatophores when prepared from phototrophs) since the membrane is oriented in the opposite direction to that in the intact cell, i.e. $BF_1$ is exposed on the outer surface. Inside-out vesicles are routinely used for investigating oxidative and photosynthetic phosphorylation, whereas right-side-out vesicles are more suited to the study of solute transport. It should be noted, however, that such vesicles are often far from being structurally perfect or homogeneous, particularly when prepared from respiratory membranes.

## Summary

Bacteria conserve energy mostly in the form of adenosine 5′-triphosphate (ATP) which they synthesize via substrate-level, oxidative (respiratory chain) or photosynthetic phosphorylation. The former is principally associated with anaerobic fermentative bacteria and is a scalar cytoplasmic process that entails the sequential formation of non-phosphorylated and phosphorylated, energy-rich intermediates. The latter two processes, on the other hand, are the major energy-conserving reactions in non-fermentative organisms, and are vectorial membrane-bound phenomena in which sequential redox reactions drive ATP synthesis via the intermediate formation and utilization of energized protons.

During respiration and photosynthesis reducing equivalents are transferred from diverse donors to higher redox potential acceptors via a highly organized sequence of redox carriers (nicotinamide nucleotides, flavoproteins, iron-sulphur proteins, quinones, cytochromes, copper proteins and molybdoproteins) which contain organic or metal redox centres. Oxygen is the most frequently used acceptor for respiration, but is occasionally replaced by alternative inorganic or organic oxidants, and organic donors are sometimes replaced by inorganic reductants.